Patients and Methods: Between 10/04 and 6/06, 24 patients (pts) with high risk/relapsed/refractory hematologic malignancies have undergone NST using a modification of our original Pt-TBI regimen. The median age was 60 years. The median number of prior therapies was 2 (range 0-6). Diseases transplanted included acute lymphoblastic leukemia (n=3), myelodysplastic syndrome (n=2), acute myelogenous leukemia (n=8), chronic lymphocytic leukemia (n=3), indolent non-Hodgkin's lymphoma (n=2), and mantle cell lymphoma (n=6). Conditioning consisted of Pentostatin 4 mg/m2 daily on day -10, -9, and -8, followed by 200 cGy TBI on day -1. Post-grafting immunosuppression consisted of cyclosporine/mycophenolate mofetil. **Results:** Transplantation was performed using mobilized progenitor cells from matched related (n=8) or unrelated (n=16) donors. Death prior to 100 days post transplant occurred in 4 unrelated donor transplants. The median nadir values for hemoglobin, neutrophil count and platelet count were 8.9 g/dl (range 7.6-13.7), 300/mm^3 (range 0-1900), and 63/mm^3 (range 9-165) respectively. Primary graft failure/autologous recovery occurred in one patient with mantle cell lymphoma. The median values for CD3+ cells and WBC at day 28 were 85% and 90% donor cells respectively. The analogous median values at day 70 were 85% and 100% respectively. One pt with a myelproliferative disorder and thalidomide as his only prior therapy experienced late graft failure despite donor lymphocyte infusions. The cumulative incidence of grade II-IV acute graft-versus-host disease was approximately 54% (14% in related versus 68% in unrelated donors, P=0.08). The probability of extensive chronic graft-versus-host disease in patients surviving beyond 100 days is 38%. The cumulative incidence of relapse at one year post transplant is 43%. The one year probabilities of event-free and overall survival are 41% and 61% respectively. **Conclusions:** This modification of our original Pt-TBI regimen continues to demonstrate fairly minimal regimen related toxicity, although as expected hematologic toxicity appears to be more significant than with our prior day -21 Pentostatin regimen. Graft versus host disease continues to be a major cause of morbidity/mortality, particularly in unrelated donor transplants. Further studies will concentrate on attempting to decrease the incidence of acute and chronic graft versus host disease through the use of T-cell depletion with in vitro alemtuzumab.

229

UMBILICAL CORD BLOOD TRANSPLANTATION FOR ADULT PATIENTS WITH CHRONIC MYELOID LEUKEMIA

Brunstein, C.[1], Setubal, D.[1], DeFor, T.[1], Weisdorf, D.[1], McGlave, P.[1], Miller, J.[1], Wagner, J.[1] [1]University of Minnesota Blood and Marrow Transplant Program, Minneapolis, MN.

Chronic myelogenous leukemia (CML) was primarily treated with HSCT until imatinib mesilate was shown to be effective and safe for patients with early chronic phase CML. However, patients who fail imatinib therapy due to disease progression or drug intolerance still require HSCT. Umbilical cord blood (UCB) has been an increasingly used source of hematopoietic stem cells for transplantation (HSCT) of patients with hematologic malignancies who lack a suitable sibling donor. We report here on 20 adult patients who underwent UCB transplantation (UCBT) for Ph+ CML at the University of Minnesota between 1998 and 2005. Patient received myeloablative (MA, n=12) or nonmyeloablative (NMA, n=8) conditioning, The median age was 46 y (r: 18-58), 12 (60%) were male, and median weight was 78 kg (r: 57-103), and 13 (65%) were CMV positive. The MA conditioning was Bu/Cy (n=2) Cy/TBI ± ATG (n=4), or Cy/Fludarabine(Flu)/TBI (n=6). The NMA conditioning was Bu/Flu/TBI (n=2) or Cy/Flu/TBI± ATG (n=6). Posttransplantation immunosuppression was CsA alone (n=1), CsA/methylprednisolone(MP) (n=5), or CsA/MMF (n=14). Eleven patients (55%) receive a double UCB graft. The highest HLA disparity of UCB units was 4/6 (n=13), 5/6 (n=5), and 6/6 (n=2). Six patients (30%) were in first chronic phase (CP1) and 14 (70%) were in accelerated phase (AP) CML. The median TNC dose infused was 2.9 × 107/kg (r:1.2-5.3) and median CD34 dose infused was 4.8 × 105/kg (r: 0.7-12.7). The median time from diagnosis to transplant was 24.5 months (r: 6.7-118.8), and the median follow-up of surviving patients was 2.9 yrs (r: 0.7-.7.0). There were no failures of neutrophil engraftment. In the MA setting median time to neutrophil engraftment was 21d (r:13-33), grade II-IV acute GVHD was 58% (95%CI, 28-88%), 1-yr transplant related mortality (TRM) 41% (95%CI, 13-69), 2-yr relapse rate 10% (95%CI, 0-26), and overall survival 58% (95%CI, 30-86). In the NMA setting median time to neutrophil engraftment was 13d (r:5-32), grade II-IV acute GVHD was 63% (95%CI, 28-98%), 1-yr transplant related mortality (TRM) 38% (95%CI, 6-70), 2-yr relapse rate 13% (95%CI, 0-34), and overall survival 50% (95%CI, 15-85). There was no statistically significant difference between MA and NMA conditioning regimens on all outcomes. In this report we show that UCB appears to be a safe and effective HSC for transplantation of patients with CML.

230

FLUDARABINE/FULL DOSE I.V. BUSULFAN CONDITIONING REGIMEN IN ALLOGENEIC PBSC TRANSPLANTATION FOR HIGH RISK PATIENTS

Chunduri, S.[1], Dobogai, L.C.[1], Peace, D.[1], Saunthararajah, Y.[1], Quigley, J.[1], Chen, Y.-H.[1], Boccuni, P.[1], Hoffman, R.[1], Mahmud, N.[1], Beri, R.[1], Hurter, E.[1], Rondelli, D.[1] [1]Section of Hematology/Oncology, University of Illinois at Chicago, Chicago, IL.

Fludarabine/ full dose busulfan (FluBu) conditioning regimen causes moderate extrahematologic toxicity and low rates of acute graft-versus-host disease (GVHD) in allogeneic hematopoietic stem cell transplantation (HSCT).

In this study we utilized this regimen in 21 adult patients with hematologic malignancies, including 15 patients (71%) at high risk of relapse (8 acute leukemia in relapse, 3 NHL in relapse, 1 NHL in CR2, 1 myelofibrosis in transformation, 2 CML-AP resistant to imatinib) and 6 patients at standard risk (3 AML in CR1, 2 CML-CP resistant to imatinib, 1 MDS). All patients were prepared with fludarabine (30 or 40 mg/m^2/day) for 4 days from d-9 to d-6 and i.v. busulfan (3.2 mg/kg/day) for 4 days from d-5 to d-2, and received an HLA matched related (n=14) or unrelated (n=7) peripheral blood stem cell (PBSC) transplant. Mean number of CD34+ cells infused was 7.3±4.0 × 10^6/kg. Thymoglobulin was added to the preparative regimen on d -3 to d-1 in 9 patients, including those receiving an unrelated HSCT. Acute GVHD prophylaxis included standard tacrolimus and methotrexate on d1, d3, d6 and d11. All patients fully engrafted but one ALL patient transplanted in relapse who recovered with leukemic blasts. Median times to ANC 0.5×10^9/L and platelet 20×10^9/L were 15d (range: 18-24) and 13d (range: 0-24), respectively. Acute GVHD grade II-IV was observed in 24% of the patients (grade III-IV 18%) and chronic GVHD in 9 of 14 evaluable patients (64%). Of 21 patients, 8 died in full relapse with bacterial or fungal infection and 2 for acute GVHD grade III and fungal infection. Median time to relapse after HSCT was 72d (range: 18-328). At a median follow-up of 447 d (range: 120-1196) for patients who are alive, the overall survival (OS) and event-free survival (EFS) are 52% and 48%, respectively. In the group at high risk the OS and EFS are both 33% at 447 d median follow-up (range: 147-834).

FluBu conditioning regimen, besides causing low transplant-related morbidity and mortality, is also effective in high risk patients receiving an allogeneic PBSC transplant from related or unrelated donors.

231

HAPLOIDENTICAL NON-MYELOABLATIVE HEMATOPOIETIC TRANSPLANT WITH SIROLIMUS BASED IMMUNOSUPPRESSION YIELDS RELIABLE ENGRAFTMENT AND MAY RESULT IN LONG TERM SURVIVAL

Claxton, D.F.[1], Creme, L.[1], Ehmann, W.C.[1], Rybka, W.B.[1] [1]Penn State Cancer Institute, Hershey, PA.

Timely availability of matched donors limits allo-hematopoietic transplant (HCT) options for many otherwise suitable patients. We have explored sirolimus (rapamycin) based immuno-

suppression after non-ablative conditioning for haploidentical (3/6 and 4/6 MHC matched related donor) blood HCT. 17 patients with poor prognosis hematological malignancies received cyclophosphamide (1gm/m2 days -7 and -6) and fludarabine (25mg/m2 days -7 through -3) with sirolimus and tacrolimus, (both adjusted to 5-15ng/ml) and methotrexate (5mg/m2 days 1,3, 6) immunoprophyllaxis. Tacrolimus was tapered between days 40-100 in patients without acute GVHD. ATG 30mg/M was also given days -1,1,3, and 5. Median age was 61. 10 patients had acute leukemias, 6 had Non-hodgkins lymphoma and one Hodgkins disease. Only 6 patients were in remission (CR) at the time of transplant, all of the other 11 having multiply pretreated active malignancy at HCT. Donor cells engrafted stably in all patients (table below). 16/17 achieved >70% donor chimerism by day 30 and 11/13 >90% by day 100. 15/17 patients developed acute (<d100) GVHD, but this was >grade 2 in only 4/17. In 1 patient GVHD developed at day 121 only after discontinuation of both tacrolimus and sirolimus. To date 2 patients remain alive free of any progression of malignancy 1216 and 1543 days post HCT. Only one of these survivors was in CR at HCT, and this individual is now enjoying a second remission more than 3 years longer than his first. 6 deaths have resulted from GVHD/Infection, while progressive malignancy (PD) has been the cause of mortality in 8 patients. One patient died of an unrelated cerebrovascular accident while free of disease or GVHD at 689 days from transplant. We conclude that, while GVHD and transplant related mortality remain obstacles, sirolimus and non-ablative conditioning allow reliable engraftment of haplodentical donor HCT with some long term survivors. These findings were in elderly and infirm patients with advanced hematological malignancy.

*Engraftment of Haploidentical Cells**

	D0	D15	D30	D60	D100
Mean Chimerism	0	0.73	0.88	0.94	0.98
Standard Deviation	0	0.38	0.19	0.15	0.05

Whole Blood Chimerism*

232

A CRITICAL ROLE OF CD100 IN ALLOGENEIC IMMUNE RESPONSES

Duran-Struuck, R.[1,2], Lowler, K.[1], Beth, W.[1], Luker, G.[3], Liu, C.[4], Kumanogoh, A.[5], Reddy, P.[1], Ferrara, J.L.M.[1] [1]University of Michigan Cancer Center, Ann Arbor, MI; [2]University of Michigan Unit for Laboratory Animal Medicine, Ann Arbor, MI; [3]University of Michigan Dept of Microbiology & Immunology, Ann Arbor, MI; [4]University of Florida College of Medicine, Gainesville, FL; [5]Osaka University, Osaka, Japan.

Sema4D (CD100) is a novel 150 kDa protein that belongs to the semaphorin family and has recently been shown to modulate autoimmunity. We tested the requirement of CD100 expression on T cells in regulating allo-immune responses. When allogeneic BALB/c stimulators were cultured with responder cells from either wild type(wt) B6 or CD100 deficient-/-B6, T cells from the CD100-/-animals showed a ten fold less expansion than the WT controls(10,800 +/- 1,230 vs. 1,600 +/- 258 cpm $p<0.01$). Consistent with the reduced proliferation, CD100-/- T cells produced less IFNγ (1890 pg/mL) compared to B6 wt Tcells (3478pg/mL) ($p<0.01$). Similar reduction in proliferation and IFN γ production was observed using anti-CD100 mAb's (data not shown). We next determined the *in vivo* relevance of CD100 expression on T cell allogeneic responses in a well characterized experimental model of GVHD. We utilized the B6($H2^b$) -->BALB/c ($H2^d$) model where the donor and recipient are mismatched at both major and minor histocompatibility antigens. Recipient BALB/c animals were irradiated with 8Gy and transplanted with 5.0×10^6 million bone marrow (BM) cells from wtB6 animals together with 0.5 million T cells from either wtB6 or CD100 -/- donors. Allogeneic recipients that were injected with CD100 -/- donor Tcells showed significantly reduced mortality, less clinical and GVHD specific target organ damage (see table). Similar benefit in the reduction of GVHD was observed with anti-CD100 mAb treatment into the recipients. When BALB/c recipient mice were challenged with the P815 ($H2^d$) murine mastocytoma cell line and received wt or CD100-/- B6 T cells, there was a significant improvement in the tumor free survival when compared to syngeneic recipients thus demonstrating preservation of graft-versus-leukemia (90% survival in allogeneic vs. 0% survival in syngeneic on day +12 post BMT $p<0.05$). We next tested the hypothesis that absence of CD100 expression on Tcells reduced the function of allogeneic APCs. When BALB/c DCs were treated with LPS and co-cultured with CD100-/- B6 T cells, they secreted less amounts of TNFa and IL12p70 compared to co-culture with wt B6 Tcells (table). Use of antiCD100 mAb's showed similar results. In conclusion, we demonstrate a novel role for CD100 in regulating *in vitro* and *in vivo* allogeneic responses using these two complementary approaches.

CD100-/- vs Wild Type Tcell in Vitro and in Vivo Outcomes

	wt T cells	CD100-/- T cells	p value
OUTCOMES			
GvHD Clinical Score (day + 60)	**4.6+/-0.8**	**1.8+/-0.2**	**<0.02**
Survival (day + 60)	**55%**	**100%**	**<0.05**
Liver Pathology (day + 60)	**17.0+/-1.5**	**9.0+/-1.3**	**<0.01**
Skin Pathology (day + 60)	**1.5+/-0.2**	**0.8+/-0.2**	**0.02**
Intestinal Pathology (day + 60)	**7.8+/-0.8**	**4.8+/-1.0**	**0.03**
CYTOKINES (pg/mL)			
IFNg serum d+14 post BMT	**584+/-148**	**286+/-97**	**<0.06**
IFNg in vitro	**3477+/-254**	**1889+/-221**	**<0.01**
DC IL-12p70 in vitro	**107+/-7**	**68.5+/-2**	**<0.01**
DC TNFa in vitro	**5930+/-123**	**4252+/-233**	**<0.01**

233

CD34 DOSE AND CHRONIC GRAFT VERSUS HOST DISEASE (CGVHD) AFFECT SURVIVAL IN ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION (ALLOPBSCT) FOLLOWING NON-MYELOABLATIVE (NM) CONDITIONING: THE VANDERBILT UNIVERSITY/NASHVILLE VA SCT PROGRAM EXPERIENCE

Ewing, G.[1,2], Giglia, J.[1,2], Vusirikala, M.[2], Ye, F.[1], Shyr, Y.[1], Chinratanalab, W.[1,2], Ruffner, K.[1,2], Kassim, A.[1], Jagasia, M.[1], Engelhardt, B.[1], Brandt, S.[1,2], Morgan, D.[1,2], Stein, R.[1], John, G.[1], Schuening, F.[1], Goodman, S.[1,2] [1]Vanderbilt University Medical Center, Nashville, TN; [2]Nashville Veterans Administration/Tennessee Valley Healthcare System, Nashville, TN.

AlloPBSCT utilizing NM conditioning is hypothesized to minimize the toxicity of myeloablative regimens while harnessing a potent graft vs malignancy effect. We sought to identify factors predicting survival in a retrospective analysis of 60 patients (pts) undergoing alloPBSCT from HLA-matched related donors between 8/00 and 8/05 at our program. All pts received 90 mg/m^2 fludarabine and 200 cGy TBI. GVHD prophylaxis consisted of CSA/MMF. The median age was 55 years (range 41-66). Male: female was 51:9. All transplants were performed for hematologic malignancies. The median number of treatments prior to transplant was 4 (range 0-8). The mean cell doses infused were 8.0 (range 3.5-16.3) x 10^6 CD34+/kg and 28.4 (range 0.9-65.9) x 10^7 CD3+/kg. Among 33 (55%) pts who became neutropenic, the median time to ANC > 500 was 21 days (range 14-49). Primary graft failure occurred in 2 patients.